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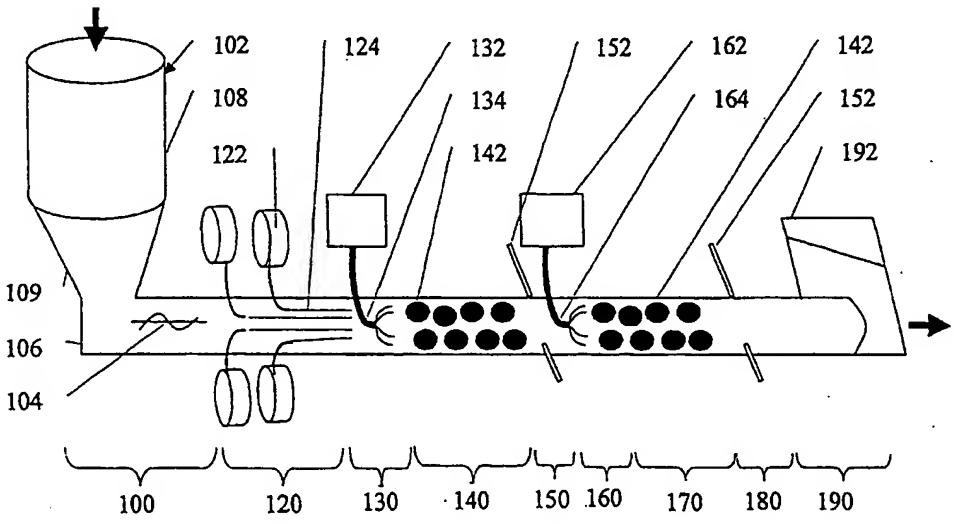

(57) Abstract

A method for preparing high strength collagen material. Collagenic material is ground and acidified with acetic acid to a pH between 3 and 4 to form a collagenic paste with a collagen content of between 3 and 15 weight percent, on a dry collagen basis. The collagen is dried, preferably by freeze-drying, to form a high strength collagen material. The collagen material has sufficient strength to be suturable. The rigid collagen material may find application as a suturable hemostatic sponge, as a rigid sponge chondrocyte cell carrier, in forming cartilaginous anatomic appendages, in forming osseous grafts, in vascular and neural regeneration, and other applications where high strength collagen is useful.

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PROCESS FOR PREPARING HIGH DENSITY MECHANICALLY RESISTANT INSOLUBLE COLLAGEN MATERIAL IN PURE AND COMBINED FORMS

Field of the Invention

This invention relates to a method of preparing high density, high strength collagen and to processes which utilize the products formed from the collagen.

Background of the Invention

In the past, collagen has been used, in the form of a soft sponge, film, gel or thread, in applications as hemostats, as surgical filler for wrinkle support, for in-vitro cell culture, or as a carrier for drug or growth factors delivery system. Formed collagen has not been used for applications where rigidity is required. The limitations in the applications for formed collagen may be due to the inadequacy of the mechanical properties of the various aforementioned mechanical forms. In particular when poured into a cavity, the existing materials do not allow proper filling of the space and cannot maintain the shape of the cavity when a low pressure is applied from outside the cavity. There is a need for a method of forming collagen, which has high density and strength.

Huc et al. (U.S. Patent No. 5,331,092) described a process for preparing collagen to be formed into products such as hemostatic pads and sponges. The process involves grinding collagen, acidifying with acetic acid, homogenizing the collagen, molding the collagen into the form of a pad or sponge, freeze drying the pad or sponge, and crosslinking the collagen by thermal treatment. Although the formed pads and sponges have higher strength than comparable pads formed by alternative methods, the only applications were as pads or sponges, uses where only limited mechanical strength is required.

Huc et al. (U.S. Patent No. 4,814,120) described a process for preparing collagen tubes for vascular applications. The collagen was coagulated in a bath containing acetone and ammonia and was crosslinked by introducing azide groups. Although the collagen tubes were described as having sufficient strength to be used in vascular applications, no applications requiring any degree of rigidity were described.

Huc (U.S. Patent No. 5,071,436) discussed formation of sponges formed from collagen-hydroxyapatite and a glycosaminoglycan. The sponges were implanted in animals as a substitute for natural bone. The bone grew around the implanted sponges to form a bone graft. The sponges were collagen combined with hydroxyapatite, however, not solely collagen, and the application as a bone substitute was through the expectation of

osteogenesis, not from the strength of the sponge itself. Pitite (U.S. Patent No. 5,264,551) crosslinked collagen by exposing the collagen to diphenylphosphorylazide.

There is a need for a method of forming high strength collagen without the use of other chemical agents.

Summary of the Invention

One aspect of the invention relates to a method for preparing high density collagen from collagenic tissue. The collagenic tissue is ground to form a ground collagen material. The ground collagen material is acidified with a weak organic acid to form a collagenic paste, where the collagenic paste has a concentration of ground collagen material between 3 and 40% by weight, based on dry collagen. The collagenic paste is dried to form high density collagen.

Preferably, the method also includes mixing the ground collagen material and the weak organic acid. The mixing may be done by ultrasonic mixing. Advantageously, the ground collagen material is acidified to a pH of between 3 and 4. Preferably, the weak acid is acetic acid. The drying may be performed by evaporation, including freeze-drying.

The method may also include washing the collagenic tissue with a phosphate buffer, where the washing occurs after grinding step. Advantageously, the collagenic paste is molded into a shape. Alternatively, the collagenic paste may be extruded into a shape. The collagenic material may be mixed with an inert material such as polytetrafluoroethylene or titanium. Advantageously, the method may also include crosslinking the collagen. The crosslinking may be done with a treatment with a reactant including glutaraldehyde, reacting the collagen with reactants including dimethylsuberimidate, reacting the collagen with reactants including azide, reacting the collagen with a reactant including epoxide, or thermally treating the collagen. Preferably, the acidifying occurs in an ultrasonic mixing tunnel.

Another aspect of the invention relates to a method of reducing bleeding from a bleeding area. The method of reducing bleeding includes providing a rigid collagen sponge prepared by a method including grinding collagenic tissue to form a ground material, acidifying the ground material to a pH between 3 and 4, forming a collagenic paste having a collagen concentration of between 3 and 15% by weight, based on dry collagen. The method of preparing the sponge also includes mixing the paste to form a homogeneous gel, forming the gel into the shape of a sponge, and drying the gel to form the rigid collagen

sponge. The method of reducing bleeding includes placing the rigid collagen sponge on the bleeding area and holding the rigid collagen sponge in place on the bleeding area. Advantageously, the rigid collagen sponge is held in place by suturing.

Another aspect of the invention relates to a method of healing a bone wound without crushing adjacent bone. The method of healing the bone wound includes compressing a rigid collagen sponge into the wound.

Another aspect of the invention relates to a method of healing a bone wound, including compressing a rigid collagen sponge into the bone wound without crushing adjacent bone.

Yet another aspect of the invention relates to a method of creating an anatomic appendage. The method includes compressing a rigid collagen sponge into the morphology of a cartilaginous anatomic appendage. Advantageously, the appendage may be sutured. Preferably, the method also includes culturing autologous chrondrocytes in the rigid collagen sponge.

Another aspect of the invention relates to a suturable collagen sponge, where the sponge contains autologous cells, autogenous cells, or growth factors.

Yet another aspect of the invention relates to a suturable collagen sponge having a fracture point of greater than 150 centiNewtons when wetted, where the fracture point is measured by placing a 5 mm high mattress loop in the sponge and pulling the loop until the sponge fractures. The sponge also has the characteristic of swelling less than known collagen sponges when wetted and compressing less than known collagen sponges when wet.

Another aspect of the invention relates to the suturability of the collagen material The collagen sponge of the invention has a fracture point of greater than 150 centiNewtons when wetted, where the fracture point is measured by placing a 5 mm high mattress loop in the sponge and pulling the loop until the sponge fractures.

Brief Description of the Drawings

FIGURE 1 shows a schematic drawing of an apparatus for measuring the height of a wet sponge.

FIGURE 2 shows a schematic drawing of an apparatus for measuring the compressibility of a wet sponge.

FIGURE 3 shows a schematic drawing of an apparatus for measuring the force required to fracture a wet collagen sponge containing a suture.

FIGURE 4 shows a schematic diagram of an embodiment of an apparatus for forming high density collagen in accordance with an embodiment of the method of the invention.

Detailed Description of the Preferred Embodiment

The present invention describes a method for preparing collagen having high strength and high density. Further, the invention describes applications for the high strength collagen.

The invention takes advantage of a high concentration of a collagen paste to obtain, after drying, a high-density mechanically resistant collagen material. As shown below, the collagen product formed according to an embodiment of the method of the invention has superior strength and rigidity compared to products formed by conventional methods. The unexpected strength of the product formed according to an embodiment of the method of the invention allows the collagen product to be utilized in applications requiring rigidity and strength, for example, being sutured into position in the body to control bleeding. Conventional formed collagen breaks when attached to the body with sutures. The high strength and rigidity of the collagen formed according to embodiments of the method of the invention allow the formed collagen to be utilized in a wide range of applications for which conventional formed collagen is not suitable. Other applications of the high strength, rigid collagen formed by embodiments of the method of the invention are described in more detail below.

Although not wishing to be tied to a theory for the reasons for the rigidity and strength of the collagen formed according to the embodiments of the method of the invention, it is believed that the high strength and rigidity of the formed collagen are due to utilizing high concentrations of collagen in the acidified suspension prior to drying. The concentration of the collagen in the suspension of the closest prior methods is significantly lower than in the embodiments of the method of the present invention. The formed collagen produced with embodiments of the method surprisingly has greatly improved rigidity and strength compared to formed collagen produced by the conventional methods.

The main steps for the preparation of such a material are described below in a specific example. The properties of the product prepared according to an embodiment of

the method of the invention are compared to the properties of other collagen products manufactured with other processes. The collagen pad produced according to an embodiment of the method of the present invention has superior strength and rigidity compared to collagen pads produced by conventional methods.

Example 1

Preparation of A Collagen Pad With High Strength and Rigidity

A collagen pad was prepared according to the following procedure in accordance with an embodiment of the method of the invention.

Preparation of Purified Dermis

The skin of freshly slaughtered calves was washed with distilled water for 2 hours in a container. The skin was then subjected to chemical hair removal in a bath containing:

In proportion: 400 g of skin (about 30 weight % dry material)
250 ml of water
2.5 g of 60 weight % ammonium sulfide solution
3.5 g of lime

The bath was subjected to rotating agitation for 36 hours at 4 rpm. The skin was exhaustively washed with distilled water until the hair residues were completely eliminated. The dermis was isolated from the rest of the skin by a slitting operation with a rotating band saw.

Cleaning treatment

The dermis was then cleaned by placing in a bath containing (in proportion):

400 g of dermis
50 ml of water
3 g of ammonium chloride
0.5 g of sodium metabisulfite

The bath was agitated by rotation for about two hours and thirty minutes.

The salts were eliminated by two successive washings with water, of 15 minutes' duration each, with a ratio of 100 g of skin for 200 ml of water. The purpose of this step is to eliminate all of the soluble proteins, i.e., albumin and hemoglobin.

Grinding

The treated dermis was then ground and extruded through sequential grids having holes with diameter widths of 5 mm, then 3 mm and finally 1.5 mm.

Washing of the Ground Preparation

The ground preparation was washed in apyrogenic purified water at a ratio of 1 kg of ground preparation for 5 L of water. The ground preparation was separated from the supernatant by continuous centrifugation in a Rousselet centrifuge rotating machine at approximately 4000 rpm. The ground preparation was then washed with a phosphate buffer having a pH of 7.8 with stirring for one hour in a stainless steel tank with a ratio of 1 kg of ground preparation for 5 L of buffer (21.7 g per liter Na_2HPO_4 and 20.78 g per liter KH_2PO_4), followed by centrifugation. This washing was performed twice. The phosphate was eliminated in the same manner with two successive washings with apyrogenic purified water with a ratio of 5 L of water per kg of ground material. The ground preparation was stirred in the water for one hour and was separated by centrifugation.

Preparation of the Dense Collagen Gel and the Dense Collagen Composite

The pure collagen ground material was then acidified with 22 weight % acetic acid, prepared by dilution of glacial acetic acid, to bring the pH to a value between 3 and 4. The amount of acid was 5% of the weight of the collagen, on a dry weight basis. The concentration of collagen in the suspension was about 10% by weight, on the basis of dry collagen. The final concentration of the solution was about 0.08 molar in acetic acid.

Preparation of the Rigid Collagen Sponge

The collagen paste was extruded into the form of cylinders, and the cylinders were cut into various lengths and freeze-dried. The cylinders were cooled to -30° C on a plate. The plate with the cylinders was placed in a freeze-dryer, and the freeze-dryer was evacuated to 400 microbars to sublime the water in the paste. The container was then warmed to 32° C at a pressure of 400 microbars to complete the freeze-drying. The freeze-drying lasted a total of 16 hours.

The properties of the hemostatic sponge prepared by the method of Example 1 were compared with the properties of commercially available hemostatic sponges. The measurements were obtained on sponges which were wet, because hemostatic sponges are used clinically in a wet state after they are soaked with blood. At this time, there are no standard physical tests for hemostatic sponges.

In Example 2, two types of properties were measured. First, the volume changes which occurred when the sponges were wetted with saline solution (to simulate blood) were determined. The sponge prepared according to the method of Example 1 swelled far less

when wetted than the commercially available sponges. Second, the volume changes when the sponges were subjected to compressive force were also measured. The sponge prepared according to the method of Example 1 compressed far less than the commercially available sponges.

In Example 3, the ability of the sponges to hold a suture was measured. The sponge prepared according to the method of Example 1 was far superior in its ability to hold a suture compared to the commercially available sponges.

The following hemostatic sponges were used in the tests of both Example 2 and Example 3.

Table 1

Sources of Hemostatic Sponges for Testing

Sponge No.	Name/Trademark	Generic Description	Company	Address
1	GELFOAM™	Absorbable Gelatin	Upjohn	Don Mills, Ontario, CANADA
2	ACTIFOAM™	Collagen Sponge	Davol	Cranston, RI 02920
3	INSTAT™	Collagen Absorbable Hemostat	Johnson & Johnson	Arlington, TX 76004
4	AVITENE™	Microfibrillar Collagen	Davol	Cranston, RI 02920
5	Example 1	Rigid Collagen	BioComposites	Long Beach, CA 90803

The data in Example 2 demonstrate that the hemostatic sponge prepared according to the method of Example 1 swelled less on wetting than the commercially available sponges. Further, the sponge prepared according to the method of Example 1 compressed less when subjected to compressive forces when wet than the other sponges.

Example 2

Measurement of Volume Change of Hemostatic Sponges

Upon Wetting and Volume Changes Under Compressing

All of the hemostatic sponges were cut to the same size of 242 mm^2 with a height of approximately 6 mm, except for the AVITENE™ sponge. Due to the microfibrillar structure of AVITENE™, an approximate mass of material equivalent in volume to the other sponges was cut. All of the sponges were soaked in commercially available sterile saline solution for five minutes before the wet measurements were taken. All of the measurements were made at room temperature.

The dimensions of the dry sponges were measured with a standard caliper (Mitutoyo, Japan). Because the wet sponges were easily deformed, measurement in the wet state with a caliper was believed to be too subjective to be meaningful. A custom apparatus was therefore designed in order to remove as much subjective bias as possible.

The apparatus for measuring the height of the wet sponges is shown in Figure 1. A wet sponge 10 was placed in a machined polycarbonate resin (LEXAN®) cylindrical reservoir 20. The cylindrical reservoir 20 was 20 mm high with an internal diameter of 34 mm. A metallic wire (not shown) joined the inside of the cylindrical reservoir 20 with the outside. A micrometer 30 (Mitutoyo, Japan) was supported on two metal machined blocks 40 on a platform 50 above the cylindrical reservoir 20 containing the wet sponge 10. The micrometer 30 had a metal tip 32 for contacting objects to be measured. A wire (not shown) was connected to the micrometer 30, and a second wire (not shown) was connected to the wire on the cylindrical reservoir 20. The metal tip 32 of the micrometer 30 was lowered until the metal tip 32 contacted the wet sponge 10. The first contact of the metal tip 32 of the micrometer 30 on the wet sponge 10 was identified by conduction of an electrical current through the micrometer 30, through the saline solution in the wet sponge 10, and through the wire on the cylindrical reservoir 20, completing an electrical circuit containing a battery, a lamp, and a buzzer. The height of the wet sponge 10 was read from the micrometer 30 after the initial contact of the metal tip 32 of the micrometer 30 with the wet sponge 10.

The volume of the wet sponge was calculated from the height of the wet sponge 10 and the calculated area of the sponge, assuming that the percent change in each dimension of the sponge was proportional to the change in height when the sponge was wetted. The

swelling of the sponge on wetting was calculated as the percent difference between the volume of the sponge when wet and when dry.

The compression of the wet sponge 10 when subjected to a standardized weight was also measured. The compression test was performed with the same basic apparatus that was used for measuring the volume of the wet sponge 10. A micrometer 30 with a metal tip 32 was supported on a platform 50 on two metal machined blocks 40 above a machined polycarbonate resin (LEXAN®) cylindrical reservoir 20 which was 20 mm high with an internal diameter of 34 mm. A metallic wire (not shown) joined the inside of the cylindrical reservoir 20 with the outside. The metallic wire was connected with a second wire (not shown) which was connected to a light and a buzzer. The wet sponge 10 was placed in the cylindrical reservoir 20. In the compression test, a standardized weight 60 was placed on the wet sponge 10 in the cylindrical reservoir 20. The standardized weight 60 consisted of a metal cylinder 70 and a metal ball 80. The metal cylinder 70 had a receptacle 72 on the upper surface for holding the metal ball 80. The total weight of the metal cylinder 70 and metal ball 80 was 107.3 g.

The micrometer 30 was lowered until the metal tip 32 of the micrometer 30 touched the top of the metal ball 80, conducting current through the micrometer 30, through the metal ball 80 and metal cylinder 70, through the saline solution in the wet sponge 10, and through the wire on the cylindrical reservoir 20, completing the circuit and activating the light and the buzzer. The metal ball 80 on top of the metal cylinder 70 acted as a point source of contact with the micrometer 30 to minimize potential errors which could occur due to changes in orientation of a flat surface placed on top of the sponge. The percent compression was calculated from the baseline measurements on the wet sponge, the height of the metal cylinder 70 and ball 80, and the micrometer readings when the metal tip 32 of the micrometer 30 contacted the top of the metal ball 80. The volume of the wet sponge 10 was calculated from the height of the compressed sponge and the width and length of the uncompressed wet sponge.

The percent compression is defined as the volume change of the sponge when compressed by the constant weight of the ball 80 and the metal cylinder 70 (107.3 g) as compared to the volume of the wet sponge 10 without compression.

The data on percentage swelling when the sponges were wetted and the percent compression when compressed by the constant weight are shown for the five sponges in Table 2 below.

Table 2

Percent Swelling Upon Wetting and Percent Compression Under Constant Weight When Wet

Sponge No.	Description	Percent Swelling on Wetting	Percent Compression Under Constant Weight While Wet
1	GELFOAM™	-17%	82%
2	ACTIFOAM™	+14%	91%
3	INSTAT™	+7%	61%
4	AVITENE™	-62%	88%
5	Sponge of Example 1	+8%	5%

The sponge of Example 1 underwent only small dimensional changes when wetted. The sponge of Example 1 also compressed much less than the other sponges when subjected to compressive force. As shown by the data in Table 2, the sponge of Example 1 compressed 12-18 times less than the comparable sponges. The small dimensional changes when wetted and the low compressibility when wet are both desirable factors for collagen materials which are intended to be used in structural applications in the body, because changes in size upon wetting or when subjected to pressure cause deformations in the sponge that change the appearance and size of the implanted sponge.

In an additional test of the utility of the sponge prepared by an embodiment of the method of the present invention, the ability of the sponges to be sutured into place was compared by measuring the force required to fracture the sponges. As shown by the data in Example 3, the sponge prepared by the method of Example 1 is far superior in strength and ability to be sutured into place as compared to the other sponges.

Example 3

Fracture of Sponges Under Pulling Forces

The test in Example 3 was designed to measure the ability of the sponges to be sutured into place. The test measured the force required to fracture a wet sutured sponge.

The apparatus for measuring the force required to fracture a wet sutured sponge is shown schematically in Figure 3.

A piece of each of the hemostatic sponges 90 measuring 10 mm by 20 mm by 5 mm was prepared. A vertical mattress loop 92, measuring 5 mm high was placed in each of the sponges in a dry state with a chromic gut 4-0 suture and a FS2 needle. The vertical mattress loop 92 was placed approximately midway (10 mm) through the length of the sponge 90. Each sponge 90 was held in a forceps and soaked in commercial saline solution for 5 minutes. The forceps (not shown) was attached to the bench by a bracket (not shown). The end of the vertical mattress loop 92 suture was attached to a dynamometer 94 (Correx, Bern, Switzerland), and the dynamometer 94 was pulled away from the wet sponge 90 until the sponge 90 broke. The maximum force required to break each of the wet sponges was recorded by the dynamometer. The fracture points for the five wet sponges in centiNewtons are shown in Table 3 below.

Table 3

Fracture Points for Sponges in CentiNewtons

Sponge No.	Description	Fracture Point (centiNewtons)	Comments
1	GELFOAM™	NA	Not Suturable
2	ACTIFOAM™	35	
3	INSTAT™	95	
4	AVITENE™	NA	Not Suturable
5	Sponge of Example 1	900	

Two of the five tested products (GELFOAM™ and AVITENE™) were too weak to be sutured, and they could not be tested. The sponge prepared by the method of Example 1 fractured at 900 centiNewtons, 9 times higher than the INSTAT™ sponge and 26 times

higher than the ACTIFOAM™ sponge. Unlike the other tested materials, the sponge of Example 1 is suitable for suturing.

The product sold in the United States under the trademark ACTIFOAM™ is manufactured according to the process described in the patent of Huc, et al. (U.S. Patent No. 5,331,092). The preparation process described in the latter patent utilized an acidified collagen suspension of less than 2.5 weight percent collagen, compared to 10 weight percent for the suspension in the method of Example 1, both on the basis of dry collagen. The sponge prepared by the method of Example 1 is, surprisingly, 26 times stronger than the ACTIFOAM™ sponge. Further, the sponge of Example 1 underwent only small dimensional changes when wetted and compressed far less than the comparative collagen sponges. The differences in the properties of the two sponges are summarized in the data shown in Table 4 below.

Table 4

Comparison of the Properties of ACTIFOAM™ Sponge and Sponge of Example 1

Sponge	Percent Swelling on Wetting	Percent Compression Under Constant Weight While Wet	Fracture Point centiNewtons
ACTIFOAM™	+14%	91%	35
Example 1	+8%	5%	900

The sponge prepared according to the method of Example 1 therefore swelled somewhat less upon wetting than the ACTIFOAM™ sponge prepared according to the method of the patent of Huc et al. (U.S. Patent No. 5,071,436). The smaller amount of swelling when wetted is important if a sponge is, for example, implanted to fill a depression in the body. If the implanted sponge swells when wetted with blood, the swelling would cause a bulge in the area where the sponge was implanted.

The sponge of Example 1 compressed 16 times less than the sponge of Huc et al. when compressed with constant weight. If a sponge is implanted in the body and subjected to pressure, as, for example, when the body moves and the subjects the sponge to pressure, the compression of the sponge would be visible, an undesirable feature.

Finally, the sponge of Example 1 had a fracture point 26 times higher than the sponge of Huc et al. when a suture in the sponge was pulled. The fracture point of the sponge is a measure of the ability of the sponge to be sutured.

Increasing the concentration of collagen in the acidified suspension from 2.5 weight percent to 10 weight percent therefore produced a sponge which has reduced swelling when wetted, has 16 times less compression when compressed with constant weight, and is 26 times stronger. Producing a formed collagen by embodiments of the method of the present invention therefore leads to unexpected results compared to the previous methods.

Further, in the patent of Huc et al., the produced sponge was crosslinked by heat treatment. The heat treatment improved the break strength by a factor of 3.7. It is not known whether the ACTIFOAM™ sponge was heat treated or not. If the ACTIFOAM™ sponge was heat treated, the enhancement of strength by the method of Example 1 is even more dramatic, because the sponge of Example 1 was not heat treated.

The method of embodiments of the method of the invention is not limited to the method disclosed in Example 1. For example, although the method of Example 1 described a method for preparing a collagen sponge, it is to be understood that the embodiments of the method of the invention are not limited to sponges. Collagen in the form of pads, fibers, sheets, tubes, pellets, shapes in the forms of anatomic appendages such as an external ear or nose form, anchors, and other forms may also be formed by embodiments of the method of the invention. Even collagen powder may be formed by the method of embodiments of the invention.

Although the source of collagen in Example 1 was calf skin, it is believed that other forms of collagen may be used in embodiments of the method of the invention. In some embodiments, the source of the collagen is animal skin. In some embodiments, the source of collagen is from a skin of a bovine. Calf skin is an exemplary source of collagen.

The hair is removed from the skin either mechanically or by chemical treatment. Removing the hair from the skin by chemical treatment is a preferred embodiment. Removing the hair with a combination of ammonium sulfide and lime in water is an exemplary method. Any other method of removing the hair from the skin is suitable for use in embodiments of the method of the invention.

If the hair is removed by chemical treatment, the hair residues can be removed from the depilated skin by water washing. Water washing the skin also removes the chemicals from the chemical hair removal treatment.

After the hair has been removed, the dermis is preferably separated from the rest of the skin. In some embodiments, the dermis is separated from the rest of the skin by a slitting operation with a bandsaw.

The dermis is then cleaned in a bath containing ammonium chloride and sodium metabisulfite, followed by water washing to remove the chemicals in the bath. The cleaning with ammonium chloride and sodium metabisulfite removes the soluble proteins such as albumin and hemoglobin.

The cleaned dermis is ground between 0.5 mm and 6 mm, more preferably between 1 mm and 5 mm, and most preferably between 2 mm and 4 mm, and the ground dermis is washed with phosphate buffer. The phosphate buffer is used to wash the dermis and eliminate the soluble impurities such as hemoglobin, low molecular weight proteins, peptides, and enzymes. The phosphate buffer is separated from the ground dermis. In an exemplary embodiment, the phosphate buffer is separated from the ground dermis by continuous centrifugation. A Rousselet centrifuge (Rousselet Centrifuge, Inc., Bethesda, Maryland) is an exemplary centrifuge for separating the phosphate buffer from the ground dermis. The centrifugation is preferably done at a speed of at least 4000 rpm. The ground dermis is then washed with water to remove the phosphate buffer, and the rinse water is separated from the ground dermis. Centrifugation is an exemplary method for separating the ground dermis from the rinse water.

The ground dermis is then acidified with a weak organic acid to a pH of between 3 and 4. The acidification may be done in a single step, or the acidification may be done in stages. Although any weak organic acid may be used, acetic acid is an exemplary weak organic acid, because acetic acid sublimes during freeze-drying. If acetic acid is used as the weak acid, the amount of acid is preferably about 5% by weight glacial acetic acid relative to the weight of collagen on a dry basis. The final molarity of the solution is about 0.08 molar acetic acid.

The suspension of collagen and the weak organic acid are preferably mixed. The mixing may be done by any suitable method. In an exemplary embodiment, the collagen is mixed with the acetic acid by ultrasonic mixing.

The acidified collagen is then optionally formed into a desired shape. The collagen may be formed into a shape by any suitable method. In an embodiment, the acidified collagen suspension is poured into a mold. In an exemplary embodiment, the acidified collagen suspension is extruded. Although the collagen suspension in Example 1 was extruded into cylinders, the collagen may be extruded into other shapes such as a square shape, a rectangular shape, a cloverleaf shape, an oval shape, or any other suitable shape. If the collagen is extruded, the extruded collagen material may be cut into shapes of various lengths. The concentration of the collagen in the collagen suspension is preferably between 3 and 40 weight %, more preferably between 5 and 40 weight %, even more preferably between 10 and 35 weight %, and most preferably between 20 and 32 weight %, all on the basis of dry collagen.

The collagen is then optionally dried. The collagen may be dried by any suitable method, including, but not limited to thermal heating, microwave heating, evaporation, and freeze-drying. Freeze-drying is an exemplary method of drying the collagen.

A suitable apparatus and method for freeze-drying the collagen according to embodiments of the method of the present invention are described in U.S. Patent No. 4,953,299 to Gimeno, et al., herein incorporated by reference. A cycle of freeze-drying comprises three steps: 1. freezing; 2. sublimation; and secondary drying. In the freezing step, the collagen is cooled to a temperature of solidification. During the sublimation step, the water in the collagen passes directly from the solid state to vapor. The vapor is trapped as a solid on a cold wall of the apparatus. During this operation, the partial vapor pressure above the collagen must be lower than the vapor pressure of the ice at the same temperature. The collagen remains frozen, so that the shape of the collagen remains unchanged during freeze-drying. Low pressures are utilized to remove water vapor from the collagen. During secondary drying, the collagen is heated to eliminate traces of water remaining in the product. The collagen is warmed, and low pressures are utilized to remove as much water as possible from the collagen.

If the collagen is dried by freeze-drying, the collagen paste is cooled to a temperature of between -20 and -40° C. In an exemplary embodiment, the collagen paste is cooled to approximately -30° C during the freezing step. The collagen paste is then exposed to vacuum to remove the water. The collagen paste is preferably exposed to a vacuum of 300 to 600 microbars, more preferably 350 to 450 microbars, and most

preferably approximately 400 microbars. The collagen paste is then warmed under vacuum during the secondary drying step to a temperature of 20 to 35° C, more preferably to a temperature of 25 to 34° C, and most preferably approximately 30-32° C. The collagen paste is freeze-dried for a period of 10 to 36 hours, more preferably 12 to 28 hours, and most preferably approximately 16 hours.

In some embodiments, the collagen can be packed and sterilized by either electron beam or gamma radiation.

Variations on Preparation of Collagen Sponge

The method of preparation of the collagen sponge can be varied in alternative embodiments of the method of the invention. In some embodiments, inert materials are added to the collagen paste before the paste is dried or freeze dried. The inert materials can be added to increase the strength of the collagen sponge even further or to alter the properties of the produced collagen product, such as changing the conductivity or density. The inert materials can be added at any point in the process up to the point that the gel is freeze dried. In an embodiment, the inert materials are added to the collagen before the acetic acid is added.

The inert materials can be arranged randomly in the collagen. For example, polytetrafluoroethylene, sold under the trademark of TEFLON™, may be added randomly to the collagen in the form of beads. Alternatively, titanium beads may be randomly added to the collagen. In other embodiments, the inert materials are oriented in the collagen material. For example, TEFLON™ or titanium filaments, woven or nonwoven, can be added to the collagen in an oriented manner.

Cross-Linkage

In embodiments of the method of the invention, the collagen can be crosslinked in order to change the physical properties and the in-vivo resorption time of the collagen or collagen composite device. Several cross-linking methods can be used, including, but not limited to, glutaraldehyde methods, reaction with dimethylsuberimidate, azide methods, reaction with epoxide reagent, and thermal treatment.

In the glutaraldehyde cross-linking methods, the collagen is reacted with a reagent comprising glutaraldehyde, for example: glutaraldehyde and lysine; glutaraldehyde, 1,6-diaminohexane, and water soluble carbodiimide; or glutaraldehyde with microwave irradiation.

In another embodiment, the collagen is crosslinked with dimethylsuberimidate. In another embodiment, the collagen is crosslinked by reacting with an azide, for example, acyl azide or diphenylphosphoral azide.

In an embodiment of crosslinking with thermal treatment methods, the collagen is exposed to short wave length ultraviolet radiation while being subjected to dehydrothermal treatment. The ultraviolet radiation may be in the in the range of 250 to 260 nm, more preferably 251 to 258 nm, and most preferably approximately 254 nm. In another embodiment of thermal treatment, the collagen may be exposed to dehydrothermal heat drying under vacuum at temperatures of 105 to 125° C, more preferably 107 to 115° C, and most preferably 110° C for a time of 4 to 24 hours, more preferably for 6 to 20 hours, and most preferably for 8 to 15 hours.

Sterilization

As a final treatment the collagen hard sponge can be packed and sterilized by either electron beam or gamma radiation.

Characteristics of the Produced Collagen

The collagen produced by various embodiments of the method of the invention has a unique combination of properties which make it useful for the applications which are described below. For example, the percent swelling on wetting for the collagen sponge produced by the method of Example 1 was +8%, the second lowest change of any of the collagens which were tested. Second, the percent compression under constant weight of 107.3 g while wet was only 5%, 12-18 times less than the other sponges which were tested. Finally, the fracture point of the sponge of Example 1, when a 5 mm high vertical mattress suture was placed in the sponge and the suture was pulled, was 900 centiNewtons, 9 times higher than the 95 centiNewtons for the next strongest sponge. The sponge of Example 1 was the only collagen sponge which was tested which was suturable.

In embodiments of the method of the invention, sponges produced by various embodiments of the method of the invention have fracture points when wet greater than 150 centiNewtons, greater than 250 centiNewtons, greater than 500 centiNewtons, greater than 750 centiNewtons, and greater than 850 centiNewtons when a 5 mm high vertical mattress suture is placed 10 mm deep in the sponge, and the suture is pulled until the collagen sponge fractures. All of these fracture points are at least 5 times higher than the fracture points of the strongest competitive collagen sponge which was tested. Of all the collagen

sponges which were tested, only the collagen sponge produced by an embodiment of the method of the invention was able to be sutured.

The combination of properties of the formed collagen produced by embodiments of the method of the present invention such as small swelling when wetted, low compressibility when subjected to constant weight when wet, and high strength allow the formed collagen produced by the embodiments of the method of the invention to be used in unique applications, applications for which none of the other collagens which were tested are suitable, because the other collagens lack the combination of properties of the collagen produced by the various embodiments of the method of the present invention.

The Ultrasonic Mixing Tunnel (UMT)

Figure 4 shows a diagram of an ultrasonic mixing tunnel (UMT), an optional apparatus useful in preparing the collagen according to embodiments of the method of the invention. Although not utilized in the method of Example 1, the ultrasonic mixing tunnel is an apparatus which is believed to be useful for large scale production of collagen prepared according to embodiments of the method of the invention. The UMT contains 9 sections, a preparation section 100, a filament addition section 120, a first acid addition section 130, a first ultrasonic mixing section 140, a first pH measurement section 150, a second acid addition section 160, a second ultrasonic mixing section 170, a second pH measuring section 180, and a cutter section 190. Several of the 9 sections are optional. Each of the nine sections will be described in turn.

Preparation Section

The preparation section 100 comprises a mixing tank 102, a pump 104, and a preparation tube 106. The mixing tank 102 can be of any suitable shape, including rectangular, cylindrical, round, etc. The embodiment of the mixing tank 102 shown in Figure 1 has a cylindrical section 108 and a conical-shaped disengaging section 109.

The pump 104 can be any of a variety of types of pumps. A helical pump is a preferred embodiment of the pump 104. A helical pump made by Alfa Laval is a suitable helical pump for use in the UMT.

The location of the pump 104 varies, depending on the form of pump 104 which is used. In the embodiment shown in Figure 1, the pump 104 is located inside the preparation tube 106. In other embodiments, the pump 104 can be located external to the preparation

tube 106, inside the mixing tank 102, or in any location where material can be pumped from the mixing tank 102 into the preparation tube 106.

Filament Addition Section

The filament addition section 120 is an optional section and comprises one or more filament bobbins 122 which hold filaments 124. Although the filament bobbins 122 can take various forms, in one embodiment the filament bobbins 122 are spools on a spindle (not shown). The filaments 124 can be made of a variety of materials. In an embodiment, the filaments 124 are made of inert material. Polytetrafluoroethylene (PTFE), sold under the tradename TEFLON™ is an exemplary inert material for the filaments 124. Carbon fibers and titanium filaments, either woven or not, are other exemplary inert materials for forming the filaments 124.

First Acid Addition Section

The first acid addition section 130 comprises a tank (not shown) for holding acid, a first acid addition pump 132, and a first acid addition pipe 134. The first acid addition pump 132 can be can be any of a variety of types of pumps. A Milton Roy pump is an exemplary pump for use as the first acid addition pump 132. The first acid addition pump 132 is preferably made of materials that are chemically resistant to acid. Suitable materials include stainless steel, TEFLON™, carbon steel, where the acid concentrations are low enough that the acid does not corrode the carbon steel, Hastalloy, Inconel, and other metals and metal alloys which are chemically resistant to the acid.

The acid can be any suitable weak organic acid. The acid of choice is acetic acid, due to the ability of this chemical to be easily eliminated during the freeze-drying step. If acetic acid is utilized as the acid to be pumped by the first acid addition pump 132, the acetic acid is preferably at a concentration of between 10 and 50 weight %, more preferably between 15 and 30 weight %, and most preferably between 18 and 25 weight %.

First Ultrasonic Mixing Section

The first ultrasonic mixing section 140 comprises a plurality of piezoelectric crystals 142. Although the piezoelectric crystals 142 can be placed inside the preparation tube 106, in an exemplary embodiment, the piezoelectric crystals 142 are bonded to the outside surface of the wall of the preparation tube 106. The piezoelectric crystals 142 are connected to a high frequency alternating current generator (not shown).

First pH Measurement Section

The first pH measurement section 150 comprises at least one pH electrode 152. The pH electrode 152 is a glass electrode of a form well known to those skilled in the art. The pH electrode 152 measures the potential which develops as a result of the difference in hydrogen ion activity in the sample and a standard solution contained within the pH electrode 152. The potential gives a voltage which is measured by a measuring device (not shown). The measured voltage is related to the pH in the acidified collagen suspension inside the preparation tube 106.

Second Acid Addition Section

The second acid addition section 160 is similar to or identical with the first acid addition section 130 and comprises a tank (not shown) for holding acid, a second acid addition pump 162, and a second acid addition pipe 164. The properties of the second acid addition pump 162 are similar to those of the first acid addition pump 132.

The properties of the acid in the tank of the second acid addition section 160 are similar to the properties of the acid in the first acid addition section 130. Acetic acid is used in the second acid addition section 160. In some embodiments, the acid in the second acid addition section 150 is more dilute than the acid in the first acid addition section 130. The concentration of the acid in the second acid addition section 150 is in the range of 5 to 15 weight %, more preferably 7 to 12 weight %, and most preferably in the range of 10-12 weight %.

Second Ultrasonic Mixing Section

The second ultrasonic mixing section 170 is similar to or identical with the first ultrasonic mixing section 140. The second ultrasonic mixing section 140 comprises a plurality of piezoelectric crystals 142, preferably bonded to the outside surface of the wall of the preparation tube 106. The piezoelectric crystals 142 are connected to a high frequency alternating current generator (not shown).

Second pH Measurement Section

The second pH measurement section 180 is similar to or identical with the first pH measurement section 150. The second pH measurement section 180 comprises at least one pH electrode 152. The pH electrode 152 of the second pH measurement section 180 is a glass electrode similar to the pH electrode 152 in the first pH measurement section 150.

Cutter Section

The cutter section 190 comprises a cutter blade 192 for cutting the collagen paste into desired lengths as the collagen paste exits the preparation tube 106. The cutter section 190 is optional, because, in some embodiments, the collagen paste is placed into molds for sponges or other devices rather than being cut into cylinders of lengths.

Operation of the UMT

Ground collagen and phosphate buffer, with or without randomly mixed inert material, are placed into the mixing tank 102 in the preparation section 100. When an inert material is added to the ground collagen material, the dry collagen weight is defined as composite weight minus inert material weight.

The ground collagen, water, and optional inert material are pumped by the pump 104 from the mixing tank 102 through the disengaging section 109 into the preparation tube 106. In an embodiment, the output of the pump is established according to the optimum ultrasonic mixing time. The output may vary from batch to batch.

After entering the preparation tube 106, the collagen enters the optional filament addition section 120. The filaments 124 are optionally introduced into the collagen gel inside the preparation tube 106 by rolling the filaments 124 off the one or more filament bobbins 122. In an embodiment, the filaments 124 are introduced into the collagen gel so that the filaments 124 are oriented along the long axis of the preparation tube 106. Although the filaments 124 can be introduced into the collagen gel at various stages of the process, in Figure 1, the filament addition section 120 is before the first acid addition section 130. In other embodiments, the filament addition section 120 is located after the first or second acid sections 130 or 160 or just before the cutter section 190.

The collagen flows through the preparation tube into the first acid addition section 130. In the first acid addition section 130, acid is pumped from the tank (not shown) by the first acid addition pump 132 through the first acid addition pipe 134 into the interior of the preparation tube 106 to be mixed with the collagen.

Sufficient acid is added in the first acid addition section 130 to bring the pH to approximately 4 to 4.5 after the collagen and the acid have been mixed. In an embodiment, the first acid addition pump 132 is controlled by feedback from a pH measuring device in the first pH measurement section 150.

After the acid has been added to the collagen by the first acid addition pump 132, the acid and collagen are mixed in the first ultrasonic mixing section 140. The piezoelectric crystals 142 are activated by the high frequency alternating current generator (not shown). The frequency of the current from the high frequency alternating current generator may vary between 20 and 40 mega-Hertz. The mechanical vibration of the piezoelectric crystals 142 is transmitted into the tube wall of the preparation tube 106, mixing the acid and the collagen.

After the collagen is mixed with the acid in the first ultrasonic mixing section 140, the acidified collagen flows into the first pH measurement section 150. The pH of the collagen paste is measured with the pH electrode 152. The target value of the pH of the collagen paste in the first pH measurement section 150 is 4 to 4.5. In an embodiment, the pH in the first pH measurement section 150 is adjusted by adjusting the output of the first acid addition pump 132 in the first acid addition section 130 on the basis of the measured pH of the collagen paste in the first pH measurement section 150. Preferably, the output of the first acid addition pump 132 is adjusted to bring the pH of the collagen paste in the first pH measurement section 150 to the desired range of 4 to 4.5.

After the pH of the acidified collagen suspension is measured in the first pH measuring section 150, the collagen paste enters the second acid addition section 160. In the second acid addition section 160, acid is pumped from the tank (not shown) by the second acid addition pump 162 through the second acid addition pipe 164 into the interior of the preparation tube 106. Sufficient acid is added in the second acid addition section 160 to bring the pH of the collagen paste to a value of between 3 and 4 after the acid and the collagen are mixed. In an embodiment, the second acid addition pump 162 is controlled by feedback from a pH measuring device in the second pH measurement section 180.

After the collagen paste is acidified in the second acid addition section 160, the acid is mixed with the collagen in the second ultrasonic mixing section 170. The piezoelectric crystals 142 are activated by the high frequency alternating current generator (not shown). The frequency of the current from the high frequency alternating current generator may vary between 20 and 40 mega-Hertz. The mechanical vibration of the piezoelectric crystals 142 is transmitted into the tube wall of the preparation tube 106, mixing the acid with the collagen. In an embodiment, the rate of flow of the collagen from the mixing tank 102 into the preparation tube 106 controls the duration of vibration.

After the acid which was added to the collagen in the second acid addition section 160 is mixed with the collagen in the second ultrasonic mixing section 170, the pH of the collagen suspension is measured with the pH electrode 152 in the second pH measurement section 180. In a preferred embodiment, the pH in the second pH measurement section 170 is controlled at a range between approximately 3 and 4 by adjusting the acid addition rate in the second acid addition section 160 by controlling the output of the second acid addition pump 162 through feedback from the pH electrode 152 in the second pH measurement section 80.

After the pH of the collagen suspension in the preparation tube 106 has been measured in the second pH measurement section 180, the collagen enters the cutter section 190. The collagen is cut into specific lengths with the cutter blade 192. The cutter section 190 is optional, because, in some embodiments, the collagen paste is placed into molds to form sponges or other devices rather than being cut into cylinders of specific lengths with the cutter blade 192.

In some embodiments, the cutter blade 192 is controlled automatically with a controller (not shown) to automate the cutting of the collagen paste into cylinders of the desired length.

Although use of the Ultrasonic Mixing Tunnel (UMT) is a preferred embodiment, it is not necessary for practice of the invention.

One major advantage of the products produced by embodiments of the method of the invention is the better capacity to keep their volume when used in surgical or physiological conditions. The collagen product can be mixed with other components, for example, chemicals, cells, biomaterials to obtain a variety of composite end products.

The products can be cross-linked to improve the stability of the product to enzymatic attacks. A variety of applications for the collagen products produced by embodiments of the method of the invention are described below.

Collagen is the most ubiquitous structural material in the human body. There are many different types of collagen, and collagen is found in a variety of fibrous forms or sheets as a result of the orientation of the elements of the helical molecule protein. Additionally, the nature of native collagen favors its use for function such as: hemostasis, cellular adhesion, promoting cellular growth, surface protection, mineralization, insulation and support. The type, form, orientation and resorption rate of the collagen differ according

to the needs of the site of use. The development of a collagen material with rigid characteristics as described herein provides a more clinically manageable product than the collagen materials available previously. By regulating the processing characteristics as described, collagen products can be artificially fabricated (in the form of fibers, sheets, tubes or pellets according to the end product demand), on a prescription basis in order to replace natural collagen matrix structures. Additional biomaterials (for example, but not limited to: TEFLON™, hydroxyapatite, fibrin, growth factors, and titanium) can be modified by prescription to enhance the artificial end product.

Hemostatic Rigid Sponge

In Example 3, it was shown that a sponge prepared by an embodiment of the method of the present invention could be sutured into place, unlike the sponges prepared by other methods. Sponges prepared by embodiments of the method can therefore be sutured into place *in situ*. This feature alone enables the clinician to direct the hemostatic material to the site of need and to hold it in that location. Modifying the resorption rate of the material by crosslinking or changing the conditions of preparation of the sponge will allow hasty removal or sustained presence of the sponge. In addition, the rigid nature of the sponge provides a material that can be compressed into a bone wound. Previously, crushing adjacent bone (which actually extends the osseous wound) or the use of the bone wax substance (which incorporates a contaminant into the wound) or the use of less efficient non-rigid collagen sponge materials were the methods available. A material that can be used in such a clinical situation is a new concept.

The ability to position and compress the rigid collagen product could serve as a hemostatic arterial plug.

Reduced hemorrhage may result in reduced tissue swelling, reduced patient morbidity, reduced resolution events, potentiate wound organization and very likely, improve healing time.

Rigid Sponge Cell Carrier

Chondrocyte transplantation to repair cartilaginous articular surfaces has been attempted with equivocal results. Existing collagen sponge materials, which are soft and friable, have been the vehicle used to transport harvested autologous chondrocytes in the past. The nature of the vehicle may very likely be the explanation for the unpredictable success of the procedure. In fact, the chondrocyte implant procedure required a periosteal

flap graft. The use of a suturable rigid collagen sponge material prepared by embodiments of the method of the invention as the transport vehicle might facilitate the surgical approach. In addition, the use of the rigid sponge material that is crosslinked to accurately extend the resorption time, may enhance the procedure outcome. Use of a rigid sponge vehicle that is suturable is a new concept.

In addition to chondrocytes, other cells, growth factors, or mediators may be transported on the rigid sponge material as a carrier. For example, epithelial cells, pancreas beta cells, leukocytes, liver cells, or any other cells may be transported on the rigid sponge material. The transported cells may be autologous or autogenous (a cell autograft or allograft). Some examples of growth factors include, but are not limited to, bone morphogenetic protein, platelet-derived growth factor, and insulin-like growth factor. The rigid sponge material with the cells or growth factors may optionally be sutured into place in the body.

With the use of a rigid collagen material, it may be possible to create an anatomic appendage. The methodology would include compressing the rigid sponge into the morphology of a cartilaginous anatomic appendage, such as an external ear or nose form. Culturing autologous chondrocytes in the appendage morphology would hopefully allow cartilage formation to replace the collagen matrix and retain the shape of the compressed sponge material. The implantation of the device subcutaneously would possibly enable epithelialization of one side of the appendage. Reversing the orientation of the device would enable complete epithelialization. This is a new concept.

Rigid Collagen Membrane Epithelial Cell Carrier

Using a rigid sponge material prepared according to embodiments of the method of the invention compressed into sheets supporting an autologous epithelial cell culture may constitute a viable artificial skin graft material. Such material may facilitate connective tissue survival subsequent to epidermal injury due to trauma (abrasion), biopsy, or burns when large surface areas are involved.

Rigid Sponge Growth Factor Carrier

Bioactive substances such as growth factors have been carried to the appropriate action site within collagen sponge vehicles. The rigid collagen sponge prepared by embodiments of the method of the invention would enable suturing the sponge directly into

place. The rigidity of the material would facilitate space maintenance. Titrating the crosslinking would enable regulating the resorption to accommodate maximum bioactivity.

Vascular and Neural Regeneration

Using a rigid sponge material prepared by embodiments of the method of the invention in the shape of a tube, within an endothelial culture system, it may be possible to encourage endothelial cells to spread across the luminal surface. New technology, regarding laminin products may assist this process. Grafting this collagen tube in the place of donor radial arteries harvested for coronary artery grafts would test the feasibility of the arterial graft regenerative potential.

Using a highly compressed rigid sponge material prepared by embodiments of the method of the invention in the shape of a tube may be useful in connecting the loose ends of severed nerves in order to encourage nerve regeneration.

Osseous Graft

A rigid collagen sponge prepared by embodiments of the method of the invention which does not collapse when moistened, maintains its form in filling a void, and is suturable, may be an effective way to potentiate osseous regeneration. Indications for this utilization would include (but not limited to): avulsion bone wounds, cranial expansion procedures, and dental alveolar ridge extensions.

Injectible Rigid Collagen

Pellets of the freeze-dried rigid collagen sponge prepared according to embodiments of the present invention can be moistened and forced through a syringe under pressure to expand the tissues around a sphincter (urethral or anal). A crosslinked rigid material should retain the sphincter pressure and encourage fibroblastic infiltration and collagen remodeling initiated from the adjacent natural tissues.

Similarly, pellets of collagen prepared according to embodiments of the method of the invention could be used to reconstruct the connective tissues in laryngeal rehabilitation.

Suture Anchors

Non-invasive surgery of the joints has increased applications but also increased demands. Suturing is sometimes impossible unless an anchor exists. The rigid collagen sponge prepared according to embodiments of the method of the invention can be shaped in order to accommodate this particular need.

Composite Materials

Combining the rigid collagen sponge prepared according to embodiments of the invention with other biocompatible materials may enhance some, or all, of the aforementioned application uses. All of the following are new concepts.

One such example, would be the creation of an artificial orthopedic ligament graft. Although artificial anterior cruciate ligament grafts have been attempted in the past (with little success), combining the rigid collagen sponge material processed into a woven PTFE (TEFLON™) tubular shape may solve the wear problem exhibited by the TEFLON™ graft alone. This composite, if anchored properly, would provide initial strength to allow immediate function and at the same time encourage collagen remodeling, resulting primarily in a replacement collagen tissue. It may be desirable to utilize an electrical field during reconstitution to favor fiber orientation that is typical of the natural ligament and tendon anatomy. Suggestions for surgical placement may include tunneling through the head of tibia leaving an osseous cortex to provide anchorage.

Rigid collagen tubes with TEFLON™ supporting walls may be helpful for arterial and neural grafting purposes. This may enhance the suturable nature of the material.

Rigid collagen sponges fabricated around TEFLON™ mesh may be helpful for hernia repairs. This may enhance the suturable nature of the material.

Combining the rigid collagen sponge with fibrous or beaded TEFLON™ may create an orthopedic anchoring device, to facilitate orthopedic repair.

It is to be understood that the above described methods and applications are merely illustrative of applications of a number of preferred embodiments and are not intended to limit the scope of the invention to the particular forms set forth, but on the contrary, it is intended to cover such alternatives, modifications and equivalents as may be included within the spirit and scope of the invention as defined by the claims.

WHAT IS CLAIMED IS:

1. A method for preparing high density collagen from collagenic tissue comprising:

grinding said collagenic tissue to form a ground collagen material;

acidifying said ground collagen material with a weak organic acid to form a collagenic paste, wherein said collagenic paste has a concentration of ground collagen material between 3 and 40% by weight, based on dry collagen; and

drying said collagenic paste to form high density collagen.

2. The method of Claim 1, wherein said collagenic paste has a concentration of ground collagen material between 5 and 40% by weight, based on dry collagen.

3. The method of Claim 1, further comprising mixing said ground collagen material and said weak organic acid.

4. The method of Claim 3, wherein said mixing comprises ultrasonic mixing.

5. The method of Claim 1, wherein said acidifying comprises acidifying said ground collagen material to a pH of between 3 and 4.

6. The method of Claim 1, wherein said weak organic acid is acetic acid.

7. The method of Claim 1, wherein said drying is performed by evaporation.

8. The method of Claim 1, wherein said drying comprises freeze-drying.

9. The method of Claim 1, further comprising washing said collagenic tissue with a phosphate buffer, wherein said washing occurs after said grinding.

10. The method of Claim 1, further comprising molding said collagenic paste into a shape.

11. The method of Claim 1, further comprising extruding said collagenic paste into a shape.

12. The method of Claim 1, further comprising mixing said collagen material with an inert material.

13. The method of Claim 11, wherein said inert material is selected from the group consisting of polytetrafluoroethylene and titanium.

14. The method of Claim 1, further comprising crosslinking said collagen.

15. The method of Claim 10, wherein said crosslinking comprises a treatment selected from the group consisting of reacting said collagen with reactants comprising glutaraldehyde, reacting said collagen with reactants comprising dimethylsuberimidate,

reacting said collagen with reactants comprising azide, reacting said collagen with a reactant comprising epoxide, and thermally treating said collagen.

16. The method of Claim 1, wherein said acidifying occurs in an ultrasonic mixing tunnel.

17. A method of reducing bleeding from a bleeding area comprising:

providing a rigid collagen sponge formed by a method comprising:

grinding collagenic tissue to form a ground material;

acidifying said ground material to a pH between 3 and 4, forming a collagenic paste having a collagen concentration of between 3 and 15% by weight, based on dry collagen;

mixing said paste to form a homogeneous gel;

forming said gel into a formed gel having a desired shape; and

drying the formed gel to form the rigid collagen sponge;

placing said rigid collagen sponge on said bleeding area; and

holding said rigid collagen sponge in place on said bleeding area.

18. The method of Claim 17, wherein said holding comprises suturing said rigid collagen sponge to said bleeding area.

19. A method of healing a bone wound without crushing adjacent bone, comprising:

providing a rigid collagen sponge formed by a method comprising:

grinding collagenic tissue to form a ground material;

acidifying said ground material to a pH between 3 and 4, forming a collagenic paste having a collagen concentration of between 3 and 15% by weight, based on dry collagen;

forming said paste into a formed paste having a desired shape; and

drying said formed paste to form the rigid collagen sponge; and

compressing said rigid collagen sponge into the bone wound.

20. A method of healing a bone wound comprising compressing a rigid collagen sponge into the bone wound without crushing adjacent bone.

21. A method of creating an anatomic appendage comprising:

compressing a rigid collagen sponge into the morphology of a cartilaginous anatomic appendage.

22. The method of Claim 21, including suturing the appendage to a patient's body.

23. The method of Claim 21, further comprising culturing chondrocytes in said rigid collagen sponge.

24. A material comprising a suturable collagen sponge and at least one substance selected from the group consisting of autologous cells, autogenous cells, and growth factors..

25. A collagen sponge having a suturability strength more than five times greater than that of known collagen materials.

26. The sponge of Claim 25 which has the characteristic of swelling less than known collagen sponges when wetted and which compresses less than known collagen sponges when wet.

27. A suturable collagen sponge having a fracture point of greater than 150 centiNewtons when wetted, wherein said fracture point is measured by placing a 5 mm high mattress loop in the sponge and pulling said loop until said sponge fractures, and which has the characteristic of swelling less than known collagen sponges when wetted and which compresses less than known collagen sponges when wet.

28. A suturable collagen sponge having a fracture point of greater than 150 centiNewtons when wetted, wherein said fracture point is measured by placing a 5 mm high mattress loop in the sponge and pulling said loop until said sponge fractures.

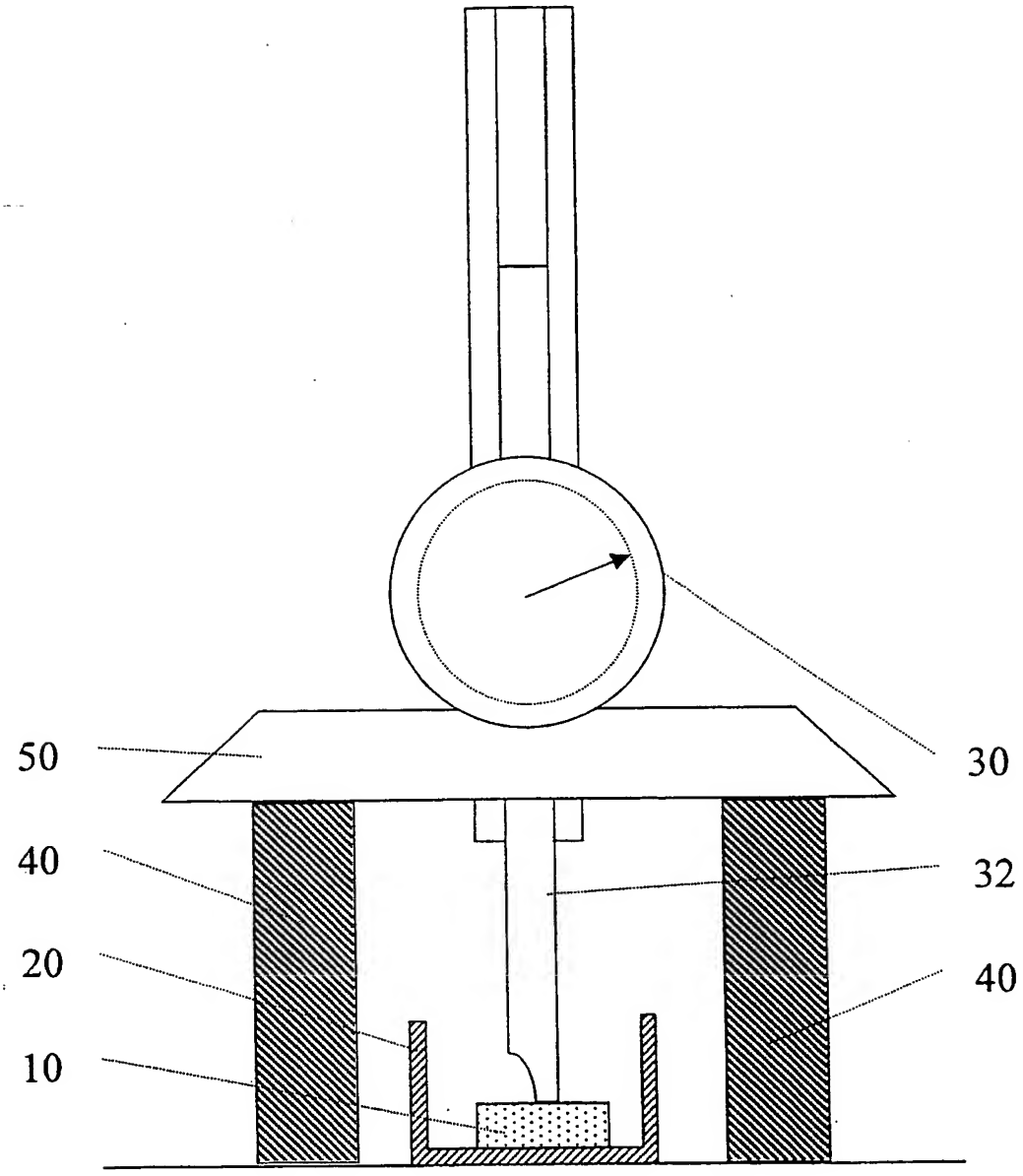

FIGURE 1

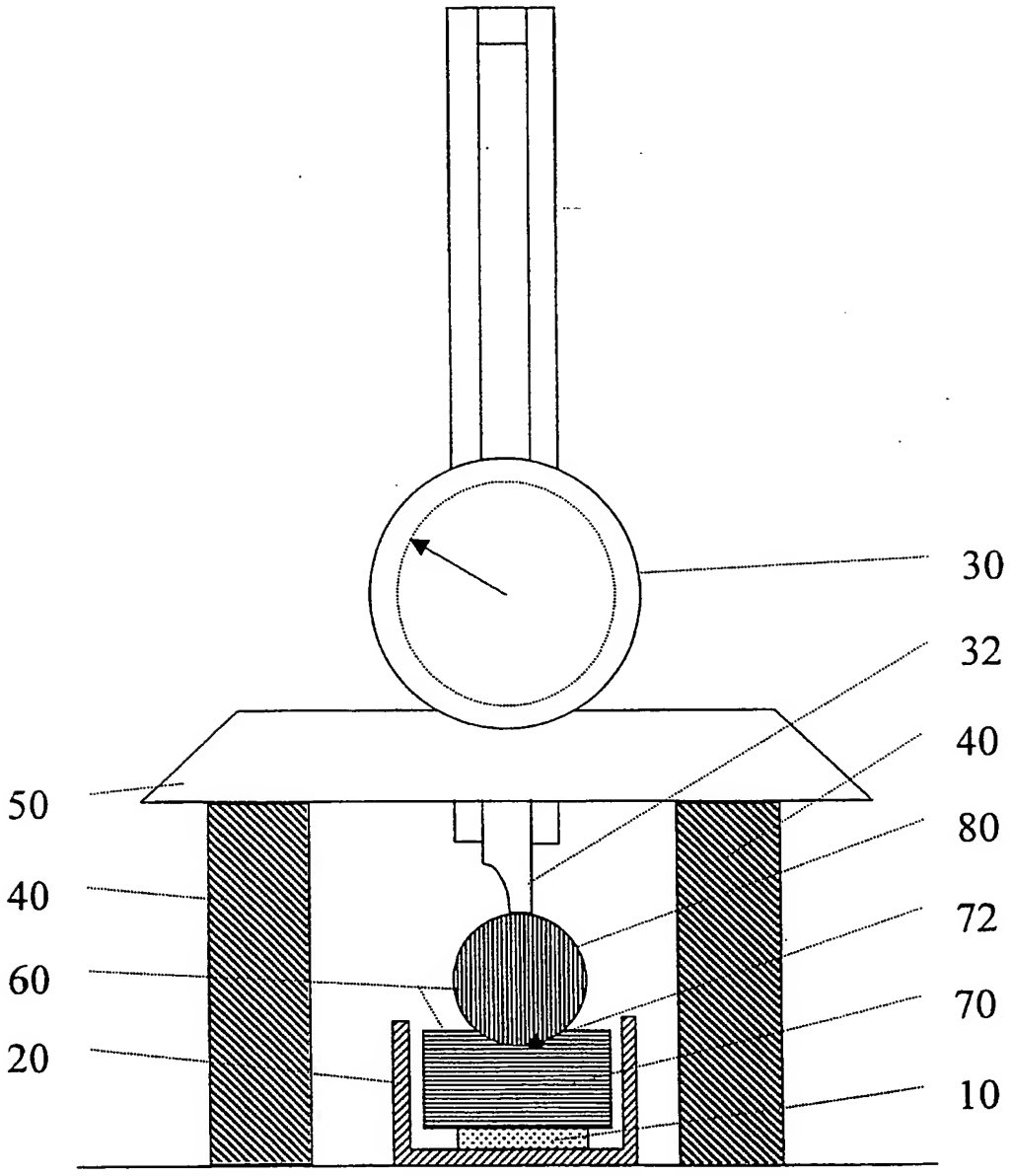

FIGURE 2

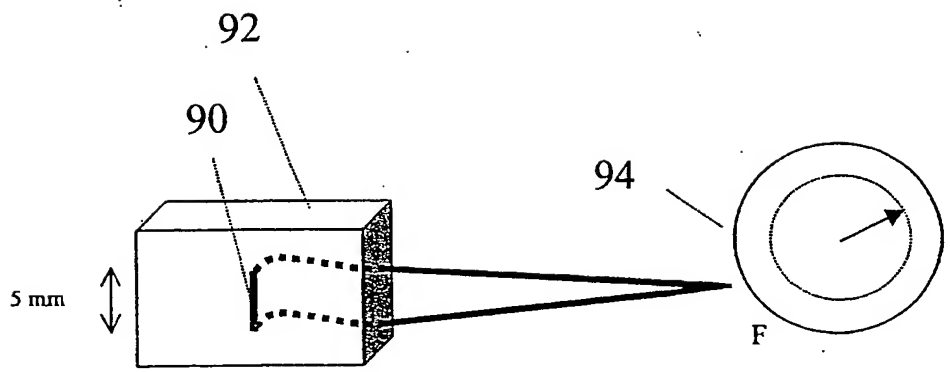

FIGURE 3

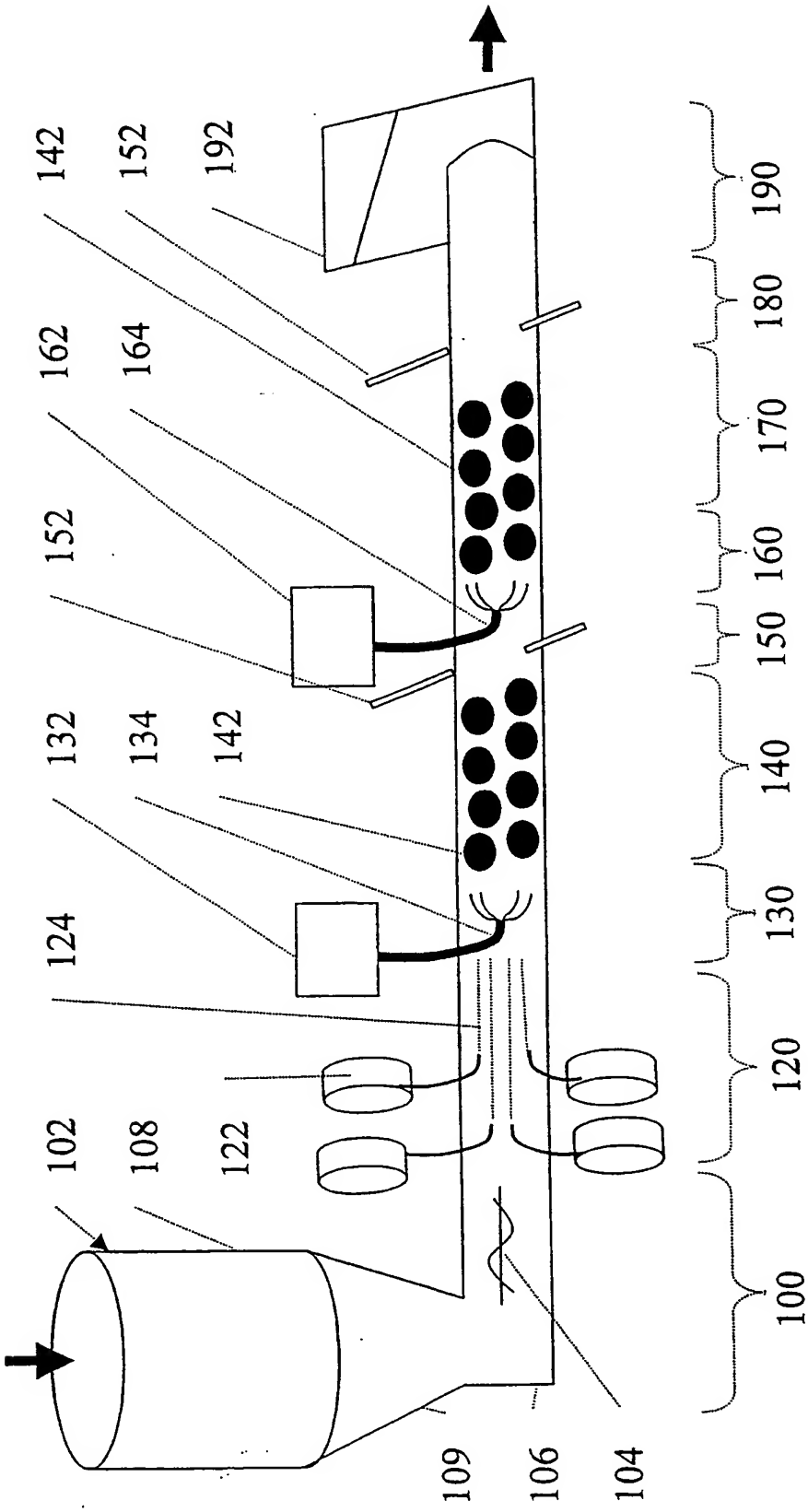
142
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FIGURE 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/27241

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C08L89/06 A61L15/32 C08J9/28 A61L27/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C08L A61L C08J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 273 705 A (TADAAKI KATO) 16 June 1981 (1981-06-16) see the whole document ---	1-10
X	US 4 412 947 A (GHEORGHE CIOCA) 1 November 1983 (1983-11-01) see the whole document ---	1-10
X	US 5 206 028 A (SHU-TUNG LI) 27 April 1993 (1993-04-27) column 4, line 32 - line 44 ---	24-28
X	US 3 587 586 A (RICHARD L. KRONENTHAL) 28 June 1971 (1971-06-28) column 2, line 8 - line 14 --- -/--	24-28

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 March 2000	23 03 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lensen, H

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/27241

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 466 462 A (ARTHUR L. ROSENTHAL ET AL.) 14 November 1995 (1995-11-14) column 3, line 6 - line 13	24

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In' ational application No.
PCT/US 99/27241

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 17-23
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

mation on patent family members

International Application No
PCT/US 99/27241

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